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Polypodium vulgare Plants Sporulate Continuously in a Non-Seasonal Glasshouse Environment

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ABSTRACT.—In their natural environments pteridophytes usually have regular sporing periods, the onset of which is triggered by the interaction of climatic and nutritional factors. Little, however, is known about what changes there may be in the sporing behaviour of a fern when it is transferred from its natural habitat to an artificial environment, such as a glasshouse. We recorded sporing behaviour in relation to vegetative growth in two genetically matched populations of *Polypodium vulgare*. One population was placed in a controlled-climate glasshouse, the other was left outside. The recruitment of new fronds was significantly higher in the indoor population than in the outdoor population. The indoor population also maintained a high proportion of actively sporing fronds throughout the winter. There was no net recruitment of new fronds in the outdoor population during the winter and early spring. Some elements of the glasshouse environment, probably the enhanced light and temperature, induced continuous sporing in this fern. Considering the ever-increasing interest in ferns as ornamental plants, and the growing body of evidence of toxic and allergenic effects caused by fern spores, this kind of sporing behaviour may have implications for human health.

Ferns in their natural environments usually have regular and predictable periods of spore production and release. In the temperate zones and the seasonal tropics they tend to release their spores towards the end of the growing season. In the wet tropics, where the growing season is much longer or even continuous, initiation of new fronds and maturation of older fronds take place throughout the year (Page, 1979). Most ferns have been said to show very little fluctuation in annual spore output with variations in climate, in contrast with good and bad seed years in angiosperms and conifers (Page, 1979). This does not apply to all fern species. Page (1976) pointed out that for *Pteridium aquilinum* (bracken) the spore yield can vary widely between different years. Similarly, Steeves (1959) noted that, for *Osmunda cinnamomea,* a hot dry summer is usually followed by a high degree of fertility in the following spring, whereas a cooler moister summer leads to reduced fertility. Furthermore, the onset of the reproductive phase in fern sporophytes can be demonstrated to be regulated by the interaction of several factors, such as light exposure, temperature and the nutritional status of the plant.

Field observations have suggested that the onset of the reproductive phase in ferns (as in many flowering plants) is induced by particular photoperiods, but the evidence so far published is scanty (Wardlaw and Sharma, 1963). Experiments carried out by Wardlaw and Sharma (1963) indicated that there is a more or less direct relationship between active photosynthesis and/or photoperiodic perception in the expanded leaves and the induction and development of sori in the next inner leaves of *Dryopteris austriaca.* Harvey and

Caponetti (1972), however, demonstrated that increasing light intensities inhibited sporophyll differentiation in *Osmunda cinnamomea*. Maximal initiation of sporangia occurred in total darkness in this species, so it may be that green and non-green spored ferns respond to light in different ways. There may be doubts about the importance of photoperiodic induction, but in determining the extent of the fertility in ferns, photosynthetically available radiation and the duration of exposure to light are probably of major importance. Steeves (1959) compared the incidence of fertility between *O. cinnamomea* plants in heavy wood and open areas and found a greater incidence of fertility in the latter plants. Conway (1957), Dring (1965) and Page (1976) suggested the same property in *Pteridium* (bracken), in which they found a gradual decrease in fertility with increasing degree of shade, although vegetative growth in the latter may be little impaired. The enhancement of sporogenesis by high light has recently been confirmed in experiments conducted with clones of bracken grown in high and low levels of photosynthetically available radiation (Wynn et al., 2000).

Temperature also plays a role in the onset of the reproductive phase as shown by Labouriau (1958). He found that initiation of sporangia was stimulated by exposure of the developing outermost set of *Osmunda claytoniana* fronds to a temperature of 26°C; plants kept at a lower temperature remained sterile. Similar trends were reported in *Pteridium* by Sheffield (1996) and Wynn et al. (2000).

Allsopp (1964, 1965) suggested that nutritional conditions, particularly carbohydrate supply, appear to be of greater importance for the induction of sporangia in pteridophytes than photoperiodic or similar stimuli. Several studies indicate that the nutritional status of the plant is indeed of great importance for the initiation of the spore-productive stage (Wardlaw and Sharma, 1963). Goebel (1887, 1905, 1908) and Atkinson (1896) concluded, from experiments with Onocleoid ferns, that if carbohydrate supplies are inadequate, developing leaves tend to remain in the vegetative state. Goebel (1928) found that immature sporophylls of certain ferns developed as vegetative fronds when the sterile fronds of the plant were removed. This has been repeated in numerous fern species (e.g. by Labouriau (1958), Wardlaw and Sharma (1963), and Steeves and Wetmore (1953)), who thus linked induction of sporogenous tissue to carbohydrate supply. Sussex and Steeves (1958) cultured excised leaf primordia of *Leptopteris hymenophylloides, Todea barbara* and *Osmunda cinnamomea,* and found that high sucrose concentrations in the medium was essential for the inception and early development of sori and sporangia in those species. They also showed that an increased supply of inorganic nitrogen promotes the onset and extent of fertility in *T. barbara*. According to Wardlaw and Sharma (1963), there is a positive relationship between the amount of photosynthesising leaf surface and the induction of fertility.

It is apparent from the above that we have some limited knowledge of what triggers sporing in ferns in natural conditions. The sporing behaviour of ferns transferred from their natural habitat to an artificial environment, e.g. a glasshouse, however, remains largely unexplored. Considering the ever-increasing

interest in ferns as household and garden ornamentals (Gress, 1996) and the fact that fern spores may cause adverse health effects in humans (Simán et al., 1999), more knowledge in this field is urgently required.

The aim of the current experiment was to compare the reproductive performance of *Polypodium vulgare* plants placed in either a seasonal outdoor environment or a constant high-temperature and high-light glasshouse environment.

Material and Methods

Polypodium vulgare plants were collected in March 1998, from stone walls along the south-east side of road B4403, running along the south-east shore of Llyn Tegid (Bala Lake) (N 52°53′, W 3°38′), Wales, U.K. *Polypodium* was chosen as it represents a widespread genus, including a broad range of horticultural favorites, such as *P. amorphum, P. cambricum* and *P. interjectum,* of similar morphology and life history (Mickel, 1994).

The plants were potted in commercial potting compost within a day of collection. Lengths of rhizome were split into two equal parts (i.e. bearing the same number of fronds in each of the two pots in a pair), and each was placed in one pot. In this way 64 pots were prepared, i.e. 32 pairs of pots containing clones. The length of the potted rhizomes varied from 1 to 5 cm, but was much the same within each pair. In order to minimise the impact on the results of the growth of any apical meristems, only median pieces of rhizome were used. All plants were allowed a six-month settling-in period (mid-March to mid-September) outdoors, after which one pot of each pair was left outdoors, to the north-east of a glasshouse in the Manchester University Experimental Grounds, Manchester, U.K. These were the "outdoor population". The other group of the plants was put inside a glasshouse (mean day temperature: 28°C, range 20–38; mean night temperature: 15°C, range 11–27; photosynthetically available radiation: c. 110 μmol m^{-2} s^{-1}), at the aforementioned Experimental Grounds. These were the "indoor population". At the start, the total number of fronds in each population was very similar. No plants were given any additional nutrients during the course of the experiment. Those outside were subject to ambient rainfall, those inside were watered regularly. During the settling-in period all fronds in 14 pots, seven in each population, died. Eight of the 14 pots belonged to matched pairs, so the aim of ensuring a genetic similarity between the two populations was still met to a high degree.

Weekly records were taken of the number of fronds in each pot with a) no sori, b) immature (green) sori, c) sporing (yellow-orange) sori and d) empty (brown) sori, from the beginning of October 1998 until June 1999 for the indoor population. The outdoor population was recorded until the end of its growing season in mid-September 1999.

The proportions of recruited fronds during the experimental period by the two populations were compared with a χ^2 test. The differences in numbers and proportions of fronds of each developmental stage in the two populations were compared numerically.

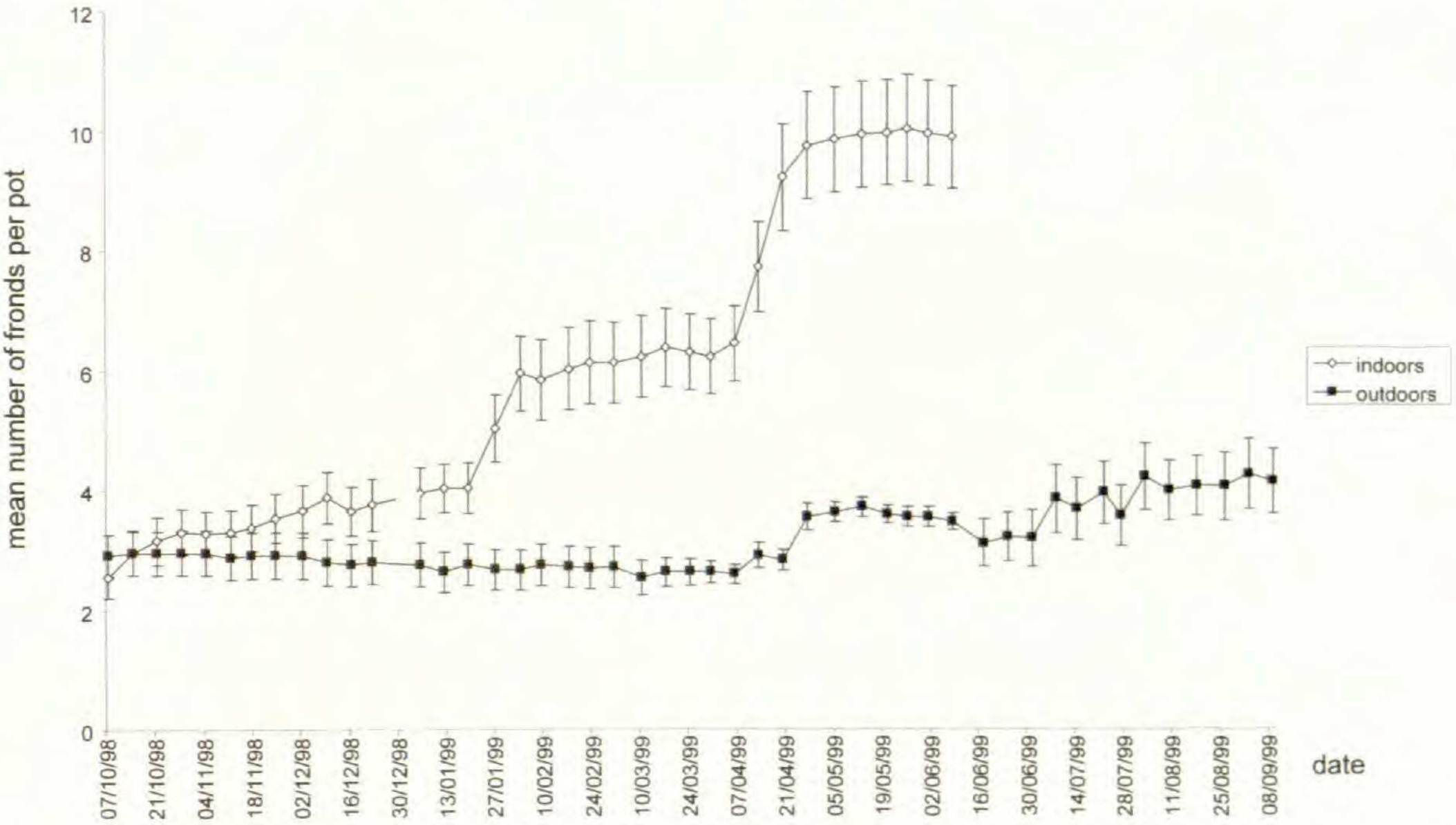

FIG. 1. Changes in the mean number of fronds per pot in two genetically matched populations of *Polypodium vulgare*. The indoor population was placed in a controlled-climate greenhouse (mean day temperature: 28°C, mean night temperature: 15°C, photosynthetically available radiation: ca 110 μmol m^{-2} s^{-1}); the outdoor population was left outside (U.K. natural weather conditions). The recruitment of new fronds in the indoor population occurred in three waves, one from October 1998 to mid-January 1999, the second from mid-January 1999 to late March 1999, and the third from early April 1999 to the end of the experiment. Error bars show standard error of the mean.

RESULTS

The recruitment of new fronds from October 1998 to early June 1999 was significantly higher in the indoor population than in the outdoor population (χ^2-test, $\chi = 127$, df = 1, $p<0.01$) (Fig. 1). During this time the indoor population increased its number of fronds more than fourfold. The recruitment of new fronds took place in three waves (Figs. 1 and 2a), each of which increased the number of fronds by a factor between 1.5 and 1.7. The outdoor population increased its number of fronds by a factor 1.2 from October 1998 to June 1999 (Fig. 1). All recruitment of new fronds in the outdoor population took place from mid-April 1999 onwards. The increase in the number of fronds in the outdoor population continued during the summer months until mid September 1999 (Fig. 3a).

The majority of the new fronds recruited in the indoor population during the course of the experiment was fertile (Fig. 2a), so the indoor population maintained a high proportion of actively sporing fronds throughout the winter. Each wave of recruitment in the indoor population began with a sudden increase in the number of initially sterile fronds; a number which decreased as sori began to appear. The proportion of fronds that remained sterile throughout the wave decreased with each wave. Thus, at the end of January 1999 the proportion of sterile fronds was 37%, in early April 1999 28% of the fronds were sterile and in early June 1999 the proportion of sterile fronds was 17%

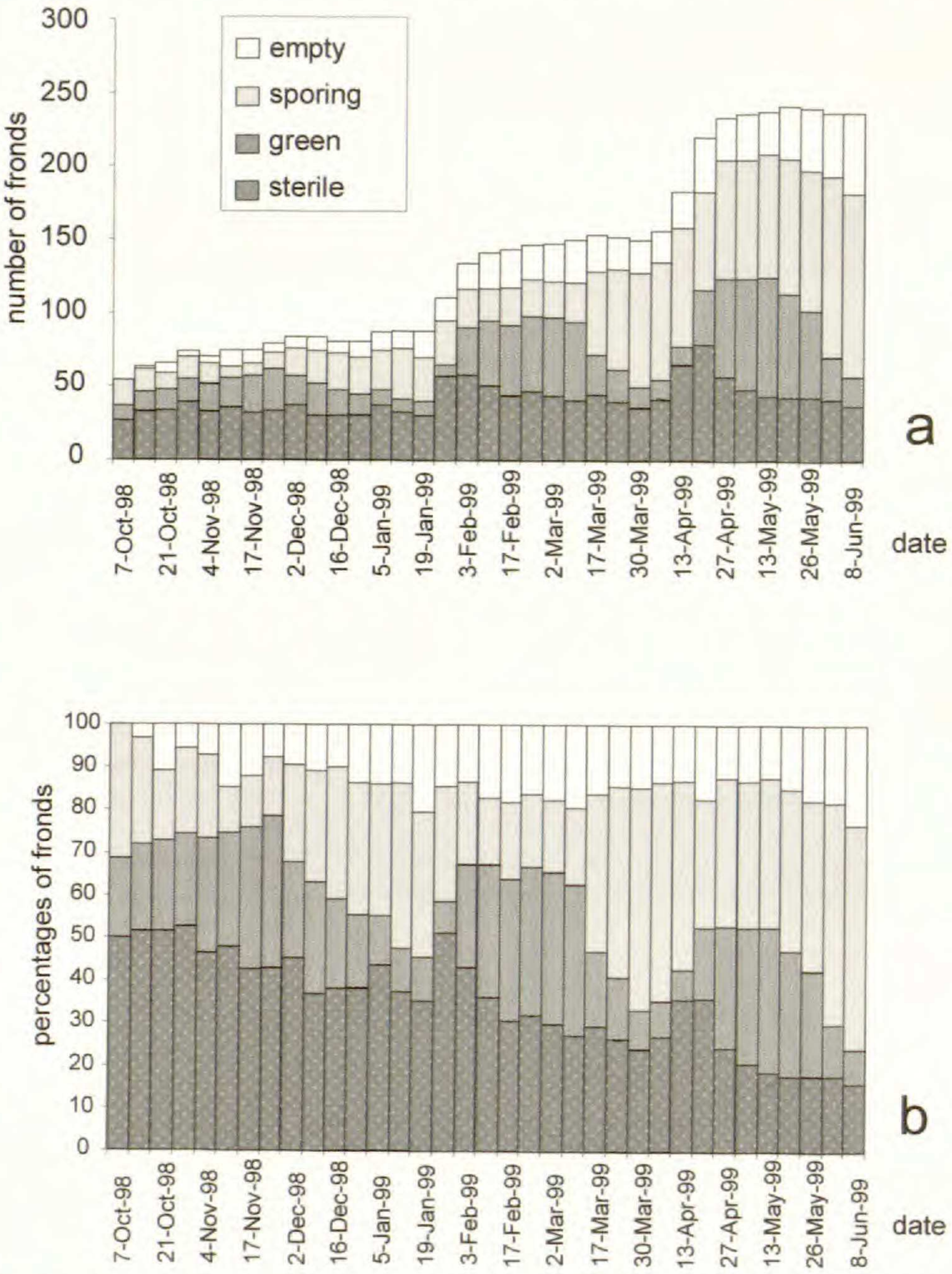

FIG. 2. Sporing behaviour in a population of *Polypodium vulgare* kept in a controlled-climate greenhouse (average day temperature: 28°C, average night temperature: 15°C, photosynthetically available radiation: ca 110 μmol m^{-2} s^{-1}) from October 1998 to June 1999. The subdivision of the bars represents i) sterile fronds and fronds with ii) green sori, iii) sporing sori and ix) empty sori, as indicated by the key in the figure. **(a)** Total number of fronds in the population (full bars) and number of fronds in each of the four groups (i.e. sterile, green, sporing, empty) for each week of the experiment. **(b)** Proportions of sterile, green, sporing and empty fronds, respectively, for each week of the experiment.

(Fig. 2b). The frond mortality represented 7.8% of the total number of fronds gained over the experimental period and was entirely due to old fronds withering and falling off.

In the outdoor population there was no net recruitment of fronds during the winter and early spring. When the new fronds started to emerge, in late April,

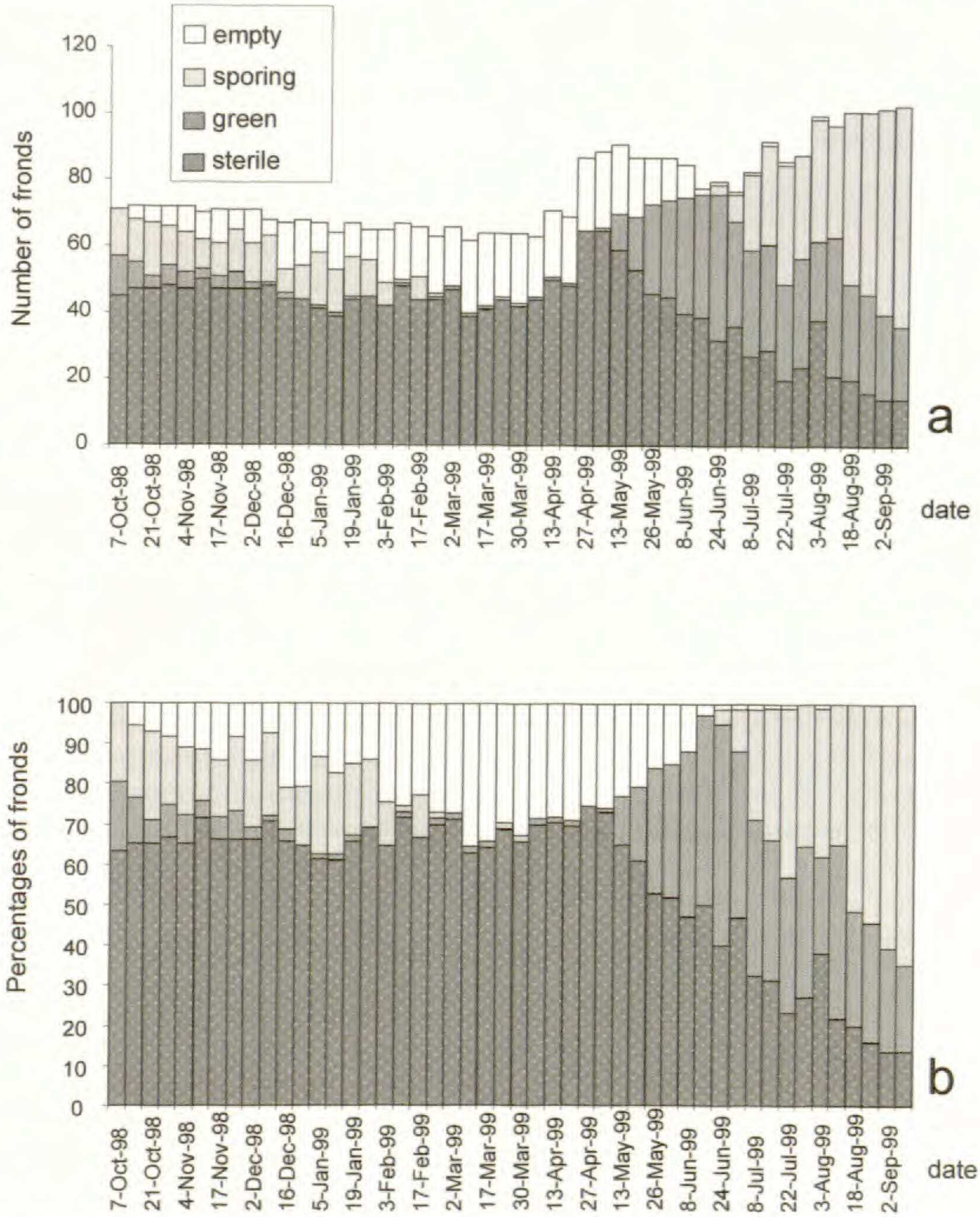

FIG. 3. Sporing behaviour in a population of *Polypodium vulgare* kept outside in the Manchester University Experimental Grounds, U.K. (natural weather conditions) from October 1998 to September 1999. The subdivision of the bars represents i) sterile fronds and fronds with ii) green sori, iii) sporing sori and iv) empty sori, as indicated by the colour code key in the figure. **(a)** Total number of fronds in the population (full bars) and number of fronds in each of the four groups (i.e. sterile, green, sporing, empty) for each week of the experiment. **(b)** Proportions of sterile, green, sporing and empty fronds, respectively, for each week of the experiment.

most of them soon turned into fertile fronds, so there was a steady increase in the number and proportion of fertile fronds over the summer (Figs. 3a and 3b). The proportion of sterile fronds decreased simultaneously so towards the end of the growing season in mid-September 1999, the proportion of sterile fronds was 17% (Fig. 3b), i.e. the same as for the indoor population at the end of its

third wave in early June 1999. The increase in number of fronds in the outdoor population was slightly impaired at two points (24/June/99 and 22/July/99) by herbivory from snails, but the fronds thus lost represented no more than 5% of the population.

Discussion

It is clear that the conditions of a warm and illuminated glasshouse stimulated the vegetative growth and spore output of the *P. vulgare* plants.

In the indoor population, the initial response to the glasshouse conditions was increased vegetative growth. At a time when the outdoor population stopped producing new fronds, the indoor population continued recruiting. Similar continuous growth has been observed in *Pteridium* grown in glasshouses (Wynn et al, 2000), but it is interesting that Thomson (2000) reports that *Pteridium* plants from four places (Honshu, Japan; Kiev, Ukraine; Bridgton, Maine, U.S.A. and Waterloo, Michigan, U.S.A.) require cold treatment (4°C) for four to eight weeks to ensure successful spring emergence of croziers when cultivated in the relative warmth of Sydney Royal Botanic Gardens (summer temp: max. 25.5°C, min. 18.2°C; winter temp: max. 16.8°C, min. 8.7°C). It seems that fluctuating temperatures warmer than those of the natural environment of a fern can have adverse effects on frond recruitment.

The majority of the new fronds emerging in the indoor population of the present study became fertile, so a high proportion of fertile fronds was maintained throughout the winter. Steeves and Wetmore (1953) concluded, after experiments with *Osmunda cinnamomea,* that the factors which determine fertility exercise their effects during the year before the leaves expanded. Assuming, in the present experiment, that each wave of recruitment created in the indoor population mimicked one growing season, we could suggest that the warm and bright indoor climate had an effect on the fertility of the second and third waves of new fronds. The proportion of fertile fronds in each of those two waves was higher than in its preceding wave. This may well be an effect of the enhanced nutritional status of the population, caused by a high production of photosynthate, which, transported as sugars to the bud primordia, might induce fertility, as suggested by Harvey and Caponetti (1972).

In its natural environment *P. vulgare* produces ripe spores from July/August. The ripening of the spores is a gradual process, occupying a period of several months. Within a single sorus some sporangia shed early and others will take longer to ripen and shed later (Wright and Wright, 1999). The persistent proportion of 10–20% sporing fronds in the outdoor population from October 1998 to March 1999 is evidence of this behaviour.

Each wave of recruitment indoors, as well as the single period of recruitment outdoors, (i.e. the growing season) increased the number of fronds by a factor of c. 1.5. This suggests that there were, at the beginning of the experiment, an equal number of dormant buds in the rhizomes of the populations and that the number of dormant buds an existing number of fronds can initiate for the next generation is restricted by something other than purely environmental

factors. This could explain the occurrence of the proportionally similar waves of recruitment.

The present study shows that by enhancing the light and temperature it is possible to interrupt the strict reproductive cycle and induce continuous sporing in a pteridophyte population. Evidence of similar behaviour has recently been obtained in another study, in which dormant *Pteridium* rhizomes produced fertile fronds within 13 weeks of being put into warm, well lit conditions (Wynn et al., 2000). Air samples taken in glasshouse and fernery environments in the UK do include ferns spores at all times of the year (e.g. Winston, 1998; Simán, 2000).

This study suggests that transfer of plants to glasshouses could benefit fern spore collectors by inducing continuous sporing in plants. There are less welcome implications of fern spore production in indoor environments, however, especially for species that do generate vastly more spores in glasshouse settings than those in natural environments. A glasshouse is an enclosed environment with little chance of biological particles being blown away by winds. This means that there is a higher risk of inhalation of fern spores in a fern-rich glasshouse than in most places outdoors. Based on the growing body of evidence of toxic and allergenic effects caused by fern spores (as reviewed by Simán et al., 1999, see also Simán et al., 2000), we suggest that some protective measures (e.g. face masks) should be taken by people who regularly work in or visit indoor fern-rich environments.

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